Regulation of intracellular pH in lungs and other tissues during hypercapnia

STEPHEN C. WOOD AND KARL E. SCHAEFER
Submarine Medical Research Laboratory, Groton, Connecticut 06340

WOOD, STEPHEN C., AND KARL E. SCHAEFER. *Regulation of intracellular pH in lungs and other tissues during hypercapnia*. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45(1): 115–118, 1978.—Using a ^{14}C-labeled DMO, ^{36}Cl, and ^{3}H method, we have determined the in vivo buffering capacity of lung, kidney, heart, skeletal muscle, and extracellular fluid (ECF) of guinea pigs during hypercapnia ($F_{I_{CO_2}} = 0.15$). After 1 days' exposure to 15% CO_2, both the relative CO_2 buffer values ($\Delta HCO_3/\Delta pH$) and the "%pH regulation" were lung > kidney > heart > ECF > skeletal muscle. For lung tissue the intracellular pH was significantly decreased only during acute (8 h) hypercapnia and had completely returned to control values after 7 days with arterial $P_{CO_2} \approx 122$ Torr. Kidney and cardiac muscle also showed ca. 100% regulation of pH at 7 days, whereas skeletal muscle and ECF showed only 80 and 70% pH regulation, respectively. The results are discussed with respect to the important (and pH-dependent) metabolic functions of the lung and kidney.

hypercapnia; intracellular pH; tissue buffering

IN TISSUES with a resting membrane potential of approximately −90 mV, the Nernst equation predicts that if the extracellular pH is 7.4, the intracellular pH will be 5.92. Likewise, for bicarbonate to be at passive equilibrium, the tissue concentration should be $^1/_{30}$ that of plasma. However, measurements of intracellular pH and bicarbonate reveal that these predicted values rarely occur, indicating the presence of some kind of "acid extruding pump" in cell membranes (15). Previous studies have shown that some tissues have much better pH regulation than others (2, 20). These differences in tissue buffering during hypercapnia may result from three factors: *1*) differences in the intrinsic noncarbonic buffer concentration; *2*) differences in the rate of formation and destruction of buffers during hypercapnia; and *3*) differences in the rate and magnitude of the transport of HCO_3^- or H^+ between the intracellular and extracellular fluid (9). For example, in rats exposed to hypercapnia, the buffering capacity of skeletal muscle exceeds that of cardiac muscle when measured in vitro; but, in vivo, cardiac muscle is buffered better than skeletal muscle (9). Since the in vitro relationship persists after exposure to hypercapnia, the in vivo reversal of buffering ability is due to the third factor, i.e., cardiac muscle has a more potent "acid pump" than skeletal muscle; or, as Lai et al. (8), have shown, skeletal muscle may even give up HCO_3^- to extracellular fluid, i.e., the in vivo buffer value is less than the in vitro value.

The relatively high in vivo buffer capacity of heart and brain (8) is adaptive considering the dependence on pH of cardiac contractility (7) and cerebral energy metabolism (4). Little, if any, information was available on the capacity of the lung or kidney to regulate intracellular pH during hypercapnia. With the strong pH dependence of most metabolic functions and the increasing awareness of important metabolic functions of the lung and kidney in mind, we have examined the in vivo buffering capacity of these tissues in comparison with cardiac and skeletal muscle in guinea pigs during chronic hypercapnia.

METHODS

Studies were conducted on male guinea pigs (Hartley strain) weighing between 400 and 600 g. Animals were housed in temperature-controlled environmental chambers. Hypercapnia was induced by maintaining an atmosphere of 15% CO_2, 21% O_2, and 64% N_2 in the chambers. Animals removed from the chambers for injection of anesthetics or isotopes or for final tissue sampling were fitted with a face mask and breathed the same gas mixture. Criteria for selecting the experimental animals and details of the exposure procedure are described by Schaefer et al. (20).

Three hours before animals were removed and killed, they were injected intraperitoneally with 2 μCi [^{14}C]5,5-dimethyloxazolidine-2,4-dione ([^{14}C]DMO, New England Nuclear), 2 μCi ^{36}Cl, and 25 μCi ^{3}H in saline (18). Blood samples were obtained in heparinized syringes from the abdominal aorta and inferior vena cava of anesthetized animals (pentobarbital, 40 mg/kg ip) breathing air (controls) or animals which had been breathing 15% CO_2 for periods of 2 h, 8 h, 1 day, 3 days, or 7 days. After blood was obtained, samples of skeletal muscle (gastrocnemius), cardiac muscle (ventricles), whole kidney, and whole lung were obtained. Excess blood, fat, and connective tissue were removed; tissues were weighed and digested for 24 h with an equivalent weight of 37.5% KOH.

Blood pH and P_{CO_2} were determined at the measured core temperature with Instrumentation Laboratories electrodes. Plasma and intracellular bicarbonate were calculated from the Henderson-Hasselbalch equation using extracellular values of $pK = 6.1$, and CO_2 solubil-

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ity coefficient = 0.03 mmol/l per Torr. To our knowledge, no measured values of these constants are available for intracellular compartments. Tissue P_{CO_2} is assumed to equal venous P_{CO_2}.

Intracellular pH was calculated from the interrelationships of the three isotopes in plasma and tissues (Beckman liquid scintillation counter) using the methods and equations of Schloerb and Grantham (21). A recent study by Boron and Roos (1) showed that intracellular pH calculated from DMO was not significantly different from direct measurements using intracellular pH electrodes.

Extra and intracellular adjustments to hypercapnia are quantified in two ways. The ratio $\Delta HCO_3/\Delta pH$, termed slykes (22), measures the "apparent CO_2 buffer value" (8); i.e., the in vivo buffer value when metabolism and exchange across membranes augments or diminishes chemical buffering. The value, "percent pH regulation" (19), calculated as ($\Delta \log HCO_3/\Delta \log P_{CO_2}$) $\times$ 100 is also used to quantitate pH regulation. This expression is particularly suitable for in vivo conditions of pH changes due to altered CO_2 tension and is linearly related to the actual pH regulation.

RESULTS AND DISCUSSION

Table 1 summarizes the extra and intracellular acid-base status of control and hypercapnic guinea pigs. The apparent CO_2 buffering value for the different tissues during both acute (2–8 h) and chronic (1, 3, 7 days) hypercapnia was usually lung > kidney > heart ≃ extracellular fluid (ECF) > skeletal muscle. A similar pattern was observed in terms of "percent pH regulation." As shown in Fig. 1, the pH of kidney, lung, and heart tissue showed almost 100% regulation of pH by 7 days. The reason for the biphasic nature of the pH regulation, with an initial sharp increase at 2 h, is unclear. The fall in bicarbonate in all tissues at 8 h indicates a superimposed metabolic acidosis, which is reversed after 24 h. Martin et al. (12) also reported a transient intracellular metabolic acidosis occurring in rats 8–24 h after exposure to 10% CO_2. In contrast to the present results, they found that extra- and intracellular bicarbonate showed maximum compensatory increases

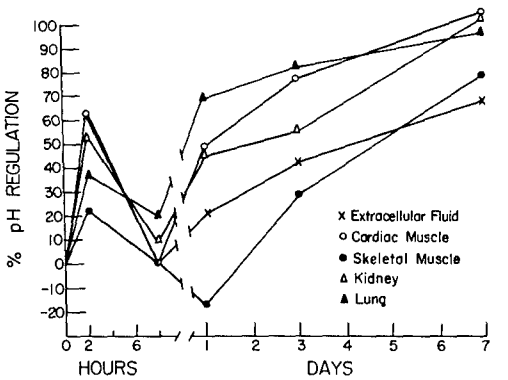

FIG. 1. pH regulation in guinea pig tissues and extracellular fluid during exposure to 15% CO_2.

TABLE 1. *Acid-base balance of extracellular and intracellular fluid of guinea pigs during acute and chronic hypercapnia*

	Air	15% CO_2				
	Control (n = 9)	2 Hours (n = 6)	8 Hours (n = 6)	1 Day (n = 6)	3 Days (n = 6)	7 Days (n = 5)
			Extracellular fluid			
P_{CO_2}, Torr	42.6 ± 2.0	116.7 ± 2.6*	121.0 ± 4.8*	116.7 ± 2.8*	125.3 ± 2.6*	122.1 ± 2.0*
pH_e	7.35 ± 0.01	7.00 ± 0.01*	6.95 ± 0.02*	7.01 ± 0.02*	7.09 ± 0.02*	7.21 ± 0.02*
HCO_3^-, mM/l	24.2 ± 0.02	29.0 ± 0.3*	24.4 ± 0.5	29.7 ± 0.8*	38.3 ± 0.8*	49.7 ± 0.2*
Slykes		13.7	0.5	16.2	54.2	182.1
% pH regulation		17.8	0.7	20.3	42.5	68.3
			Skeletal muscle			
pH_i	6.68 ± 0.04	6.52 ± 0.08	6.20 ± 0.03*	6.18 ± 0.06*	6.36 ± 0.11*	6.59 ± 0.06
HCO_3^-, mM/l	5.3 ± 0.4	9.9 ± 1.1*	5.3 ± 0.4	4.4 ± 0.7	7.3 ± 1.0	12.3 ± 0.8*
Slykes		28.7	0	−1.8	6.3	77.8
% pH regulation		62.0	0	−18.4	29.7	79.9
			Cardiac ventricle			
pH_i	6.90 ± 0.06	6.70 ± 0.11	6.44 ± 0.03*	6.66 ± 0.04*	6.77 ± 0.04	6.93 ± 0.06
HCO_3^-, mM/l	8.4 ± 0.8	15.6 ± 1.4*	8.5 ± 0.7	13.9 ± 0.9*	19.6 ± 0.3*	25.8 ± 1.0*
Slykes		36.0	0.2	22.9	86.1	580.0
% pH regulation		61.4	1.1	50.0	78.5	106.6
			Lung			
pH_i	7.13 ± 0.09	6.90 ± 0.13	6.80 ± 0.08*	7.00 ± 0.03	7.06 ± 0.03	7.12 ± 0.09
HCO_3^-, mM/l	15.6 ± 0.7	23.0 ± 0.9*	20.3 ± 0.4*	31.7 ± 0.6*	39.0 ± 0.7*	43.7 ± 1.2*
Slykes		32.2	14.2	123.8	334.3	2810.0
% pH regulation		38.5	25.2	70.4	84.9	97.8
			Kidney			
pH_i	7.22 ± 0.06	7.03 ± 0.08*	6.83 ± 0.01*	6.99 ± 0.02*	7.02 ± 0.06*	7.24 ± 0.06
HCO_3^-, mM/l	19.4 ± 0.7	33.2 ± 1.1*	21.7 ± 0.4	31.0 ± 0.5*	35.6 ± 1.3*	58.4 ± 1.1*
Slykes		72.6	5.8	50.4	81.0	1950.0
% pH regulation		53.3	10.7	46.5	56.3	104.6

Values are means ± SE. * Significantly different from control values at P less than or equal to 0.05.

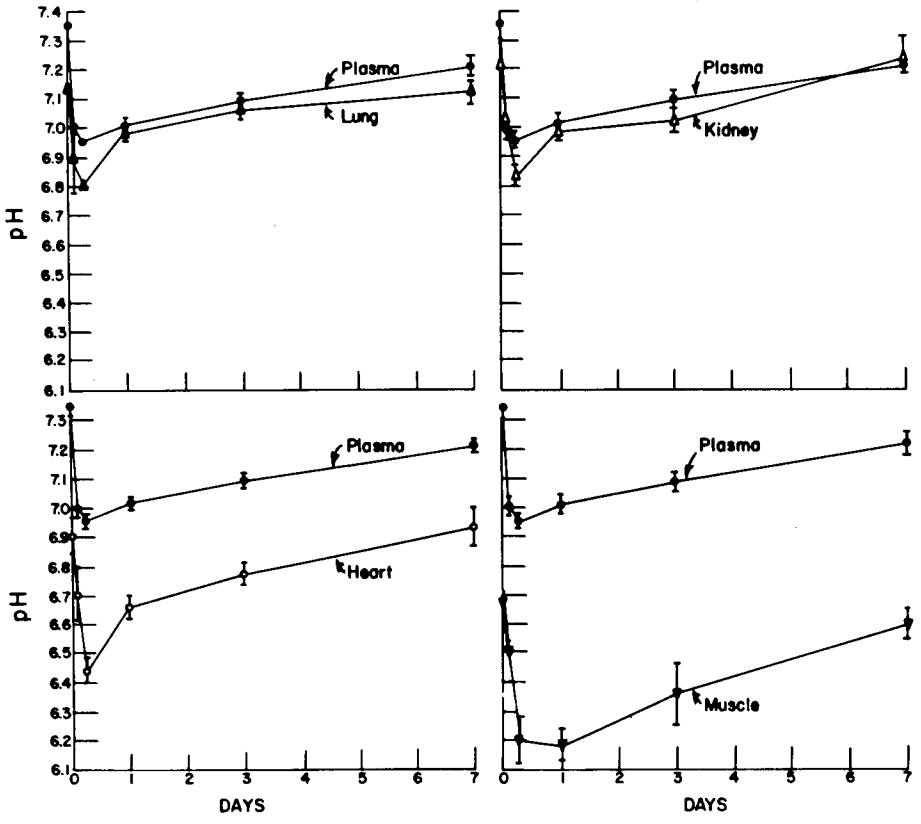

FIG. 2. Effect of hypercapnia (15% CO_2) on plasma pH and intracellular pH of lung, heart, kidney, and skeletal muscle of guinea pigs.

after 24–48 h and did not increase significantly further during the next 4 days of CO_2 exposure. In agreement with the present data, they found that, in vivo, heart muscle was better buffered than skeletal muscle, the opposite of what is found in vitro. They also studied brain and found a relative buffering value of brain > heart > skeletal muscle ≃ECF.

The only previous data on buffering capacity of lungs is an in vitro dissociation curve of dog lung (3). These data, recalculated by Fenn (6), revealed an in vitro buffer capacity of about 5 slykes, similar to that of other tissues and about one-third of the buffer capacity of blood.

The time courses of pH adjustments in tissues and ECF are shown in Fig. 2. The control values of intracellular pH of guinea pig ventricle (pH_i = 6.90) falls within the range of previous data (pH_i = 6.83 to 6.96) for dogs and rats (9, 10, 18, 21). For guinea pig skeletal muscle, control pH_i (6.68) is somewhat lower than previous data (pH_i = 6.90–7.04). The intracellular pH of guinea pig lung (pH_i = 7.13) is identical to that of dog lung at P_{CO_2} = 40 Torr (6).

It is interesting that during hypercapnia the pH_i of lung and kidney is generally the same as pH_e. However, to assess regulation, the pH_i of hypercapnic tissues must be compared with control pH_i. As seen in Fig. 2, the pH_i of heart, kidney, and lung has returned almost to control values by 7 days, whereas the pH of skeletal muscle and ECF is regulated only 80 and 68%, respectively. In the case of pH_i of lung tissue, only the pH_i at 8 h of CO_2 exposure is significantly lower than control pH_i. It should be noted that the higher CO_2 buffering value of lungs and kidneys does not prevent these tissues from being the target of histopathological CO_2 effects. The latter consisting of hyaline membrane formation in the lungs of guinea pigs exposed to 15% CO_2 (17), and kidney calcification (20). No histopathological effects were observed under the same experimental conditions in heart and skeletal muscle.

The mechanism of greater CO_2 buffer value in vivo than in vitro appears to be an active transport of bicarbonate into or hydrogen ions out of the intracellular fluid (8). The physiological significance in the case of cardiac muscle lies in the pH sensitivity of cardiac contractility (7). For kidneys, regulation of pH would protect numerous metabolic functions with pH optimal close to normal pH_i, e.g., ATPase activity (11) as well as gluconeogenesis and glycolysis (13).

In the case of lung tissue, pH regulation is essential for several key metabolic functions. For example, utilization and dependence of the lung on glucose may equal that of the nervous system (14) and, as Pérez-Díaz et al. (12) showed, glucose utilization in rat lung cells is strongly inhibited below pH 7.0. Surfactant syntheses is also pH dependent, e.g., acidemia (umbilical venous pH = 7.2) in primates causes a marked inhibition of the major pathway of lung lecithin biosynthesis in vivo (5).

Inspiration of 15% CO_2, even without concomitant hypoxia, is obviously going to stress many or all physiological systems which may interact with the present results. A previous study (20) describes the sympathoadrenal response and initial 10% weight loss of guinea pigs breathing 15% CO_2 for 7 days. In both cases, these responses were limited to the uncompensated phase (first 3 days) of acidosis. It should also be noted that the results of the present study have relevance to naturally

occurring hypercapnia, since wild guinea pigs live in burrows (22) in which inspired CO may range from 2 to 13% (8, 9, 23).

Present address of S. C. Wood: Dept. of Physiology, University of New Mexico School of Medicine, Albuquerque, N. Mex. 87131.

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